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Identification of Unique Proteomic Signatures in Allergic and Non-Allergic Skin Disease

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ABSTRACT

Background: Atopic dermatitis (AD), psoriasis (PS), and contact dermatitis (CD) are common skin diseases, characterized by barrier disruption and systemic inflammation, with unique epidermal signatures and common inflammatory pathways identified by transcriptomic profiling. This study profiled proteomic signatures in serum from subjects with AD, PS, and CD compared with healthy controls (HC).

Objective: Identify unique proteomic signatures to distinguish between inflammatory diseases with similar epidermal disruption and overlapping epithelial inflammation.

Methods: Sera from 20 subjects with moderate to severe AD, 10 subjects with CD, 12 subjects with moderate to severe PS, 10 subjects with both AD and CD, and 10 HC with no history of skin disease was analyzed using high throughput proteomic analysis that detects expression of 1129 protein targets. Protein expression was compared between disease and HC, and across diseases for statistical significance (fold change ≥ 1.5 and false discovery rate ≤ 0.05), to identify unique proteomic signatures for each disease.

Results: Complement C5a anaphylatoxin (C5A), lipopolysaccharide binding protein (LBP), C-reactive protein (CRP), ILT-4, C-C motif ligand 18 (PARC), and sialic acid-binding Ig-like lectin 14 (SIG14) were significantly modulated in all three diseases compared with HC. We identified unique signatures for AD (Immunoglobulin E (IgE), thymus- and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC)), CD (10 proteins), and PS (kynureninase (KYNU)). Proteomic profiling in subjects with both AD and CD identified additional dysregulated proteins compared with subjects with either condition alone, indicating an exacerbated inflammation reaction.

Conclusions and Clinical Relevance: Unique sera proteomic signatures may distinguish between inflammatory skin diseases despite similar epidermal barrier disruption and epithelial inflammation. This may provide insight into disease pathogenesis, diagnosis and therapeutic intervention in difficult-to-treat subjects.

Key words: Atopic dermatitis, psoriasis, contact dermatitis, clinical immunology, dermatology, biomarkers

Abbreviations used:

AD	Atopic dermatitis
BPI	Bactericidal permeability-increasing protein
BMI	Body mass index
C1R	Complement component 1r subcomponent
C5A	Complement C5A anaphylatoxin
CA6	Carbonic anhydrase 6
CAMK2B	Calcium/calmodulin-dependent protein kinase 2 beta
CCL18/PARC	C-C motif ligand 18
CD	Contact dermatitis
CD36	Cluster of differentiation 36
CRP	C-reactive protein
CXCL	C-X-C motif ligand
DBNL	Debrin-like protein
ECL	Electrochemiluminescence
FABP3	Fatty acid binding protein 3
FCH	Fold change
FDR	False discovery rate
FUT5	Fucosyltransferase 5
GCP2	Granulocyte chemotactic protein 2
G-CSF	Granulocyte colony-stimulating factor
HC	Healthy controls
HnRNP	Heterogeneous nuclear ribonucleoproteins

IDO	2,3-dioxygenase
IFN γ	Interferon-gamma
IgE	Immunoglobulin E
IL	Interleukin
IL13RA	IL13 receptor A
ILT-4	Immunoglobulin-like transcript 4
KYNU	Kynureninase
LBP	Lipopolysaccharide binding protein
LG3BP	Lectin galactoside
LILRB	Leukocyte immunoglobulin-like receptor subfamily B member 2
MCP1	Monocyte chemoattractant protein 1
MDC	Macrophage-derived chemokine
MIF	Macrophage migration inhibitory factor
MIP	Macrophage Inflammatory Protein
MOES	Moesin
PASI	Psoriasis Area and Severity Index
PERM	Myeloperoxidase
PRTN3	Proteinase-3
PS	Psoriasis
RAST	Radioallergosorbent test
RFU	Relative fluorescence units
SCORAD	Scoring Atopic Dermatitis
SD	Standard Deviation
SIGLEC14	Sialic acid-binding Ig-like lectin 14
sICAM5	Soluble intercellular adhesion molecule 5
SOMAer	Slow Off-rate Modified Aptamers

TARC	Thymus- and activation-regulated chemokine
Th	T helper
TNFRSF12A	Tumor necrosis factor receptor superfamily member 12A

INTRODUCTION

Atopic dermatitis (AD), contact dermatitis (CD), and psoriasis (PS) are common relapsing inflammatory skin diseases characterized by significant epidermal disruption, local and systemic inflammation, and impaired quality-of-life. Diseases have been extensively investigated; however, their similarities and differences are not fully understood which can complicate diagnosis and treatment.

AD affects approximately 25% of children and 10% of adults^{1,2} and is primarily associated with an increased T helper (Th) 2 inflammatory response, which modulates the epidermal barrier and innate immune response permitting enhanced penetration of allergens and pathogens.^{3,4} CD results from skin exposure to allergens or irritating chemicals and is a significant co-morbidity in approximately 40% of patients with AD, potentially because of epidermal barrier disruption that contributes to impaired protection.⁵⁻⁸ Clinical manifestations of CD can mimic AD, making it challenging to distinguish these diseases. PS affects approximately 3% of the population⁹⁻¹¹ and is characterized by recurrent episodes of red and scaly skin plaque which develop as a result of aberrant keratinocyte proliferation, differentiation, and immune infiltration. Genome-wide studies demonstrate a significant overlap between the susceptibility loci for AD and PS^{12,13} suggesting potential overlapping pathogenic mechanisms. The significant physical, psychological, and economical quality-of-life impairment associated with these diseases drives the importance of correct diagnosis and effective therapy.¹⁴⁻¹⁶

Extensive gene expression and immune cell profiling has revealed that AD is predominantly Th2/Th22 polarized, with some Th17 involvement.¹⁷ Progression of AD is associated with activation of Th2-defining cytokines, such as interleukin (IL)-4, IL-13, thymic stromal lymphopoietin, and IL-31, as well as Th22-defining cytokines, such as IL-22.¹⁸⁻²¹ Further, increased Th2/Tc2/IL-13⁺ and Th22/Tc22/IL-22⁺ cell population were observed in patients with severe AD.²² PS deviates toward Th17 and Th1 responses, with the IL-23/Th17 axis playing a critical role in the pathogenesis. IL-23 drives production of IL-17 from Th17 cells, which activates keratinocytes, and induces synthesis of pro-inflammatory chemokines, such as C-X-C motif ligand (CXCL) 1, CXCL2, CXCL3, and CXCL8.²³⁻²⁵ CD was historically considered a Th1-dominated or a mixed Th1/Th2 response; however, it is increasingly recognized that Th17 and Th22 cytokines also contribute to disease progression.²⁶⁻²⁹ A recent gene expression profiling study showed that the immune systems of patients with CD are differentially activated depending on different allergens,³⁰ suggesting overlap with other inflammatory diseases.

While gene expression analysis in skin biopsies has resulted in a greater understanding of mechanisms underlying disease and potential biomarkers,³¹ there is still a need for a non-invasive method to distinguish between inflammatory diseases. Serum protein profiling is emerging as a powerful complementary approach to genomic analysis of skin biopsies. Serum collection is minimally invasive and, combined with broad proteomic approaches, can provide access to important biomarkers of systemic inflammation. Additionally, protein concentrations may better reflect the function and dynamics of cells enabling targeted therapy to address systemic inflammation.

In this study, we utilized broad proteomic approaches to characterize serum protein profiles in subjects with active AD, CD, and PS compared with healthy controls (HC). We identified serum profiles in each subject cohort and compared these across diseases to generate unique proteomic signatures for each disease. To our knowledge, our data are the first to identify unique signatures capable of distinguishing between inflammatory skin diseases.

METHODS

Subjects

Subjects included 10 HC with no history of skin disease, 20 with moderate to severe AD (Scoring Atopic Dermatitis [SCORAD]: 66.23 ± 15.03 ; $M \pm SD$), 10 with CD, 10 with both AD and CD (SCORAD: 37.40 ± 20.33), and 12 with moderate to severe PS (Psoriasis Area and Severity Index [PASI]: 25.2 ± 10.88). Subjects were allowed to use emollients only and avoided systemic immunomodulators and phototherapy within the previous 4 weeks and topical steroids or calcineurin inhibitors within the previous 8 days. Detailed characteristics are provided in Table E1. No significant difference for genders (Chi-square test), but significant difference for age (ANOVA, p value < 0.05), was detected between different groups. Therefore age was fitted as numerical covariate for analysis (details see “statistical analysis” below).

AD subjects were further separated based on total serum IgE levels (ImmunoCAP) into intrinsic AD (IgE < 200 U/ml, $N=6$) or extrinsic AD (IgE ≥ 200 U/ml, $N=14$). Additionally, CD was confirmed in subjects by patch-testing for the 15 most common allergens of the North American Contact Dermatitis Group and petrolatum-occluded skin (as control). At 72 hours, the patch test site was evaluated for reactivity and positive reactions were clinically graded as 1+, 2+, or 3+.

These studies were conducted according to the Declaration of the Helsinki Guidelines and were approved by the institutional review board at Icahn School of Medicine at Mount Sinai (#HS 14-00436; GCO#1: 14-0863 (0001) Icahn School of Medicine) in New York. All subjects gave written informed consent prior to enrollment. Additionally, subjects confirmed the absence of comorbidities such as asthma, allergic rhinitis, diabetes, cardiovascular pathologies, or self-reported history of systematic inflammatory disorders.

Epidermal thickness

Epidermal thickness was quantified as previously described³² using computer-assisted image analysis software (ImageJ 1.42; National Institutes of Health, Bethesda, MD). The epidermal thickness measurement included all layers, including the stratum corneum.

Serum collection

Blood samples were collected using BD Vacutainer Plus plastic serum tubes. After collection, blood was allowed to clot by leaving undisturbed at room temperature for 15–30 minutes. Tubes were centrifuged within 3 hours of collection (1500 g for 10 minutes at room temperature). Sera were transferred to cryotubes and stored at -80°C until analysis.

SOMAscan™ assay

Sera were evaluated using the previously described SOMAscan™ proteomic assay (SOMAscan Assay 1.1k), which detects levels of 1129 proteins simultaneously.^{33, 34} Briefly, DNA-based Slow Off-rate Modified Aptamers (SOMAmers) were designed that specifically bind to natively-folded proteins. SOMAmers were mixed with serum samples to allow binding. Sample concentrations of 40%, 1%, and 0.005% were used to allow measurement of protein concentrations at ~8 log range. Unbound SOMAmers were then washed away. Protein-bound SOMAmers were eluted and quantified using a DNA microarray. In this way, protein concentrations in sera were transformed into a corresponding signature of DNA aptamer concentrations followed by a fluorescence signal on the DNA microarray. Values are reported in relative fluorescence units (RFU).

Serological Measurement of IgE and Inflammatory Mediators

The levels of total serum IgE were determined using ImmunoCAP - radioallergosorbent test (RAST) system (ThermoScientific; Uppsala, Sweden). Electrochemiluminescence (ECL) immunoassays were used in a multiplex format; nine biospecific antibodies were spotted at the bottom of the 96-well plate (Meso Scale Discovery; Rockville, MD) to quantify the concentrations of nine chemokines in a single sample. The chemokines measured were: Eotaxin, Eotaxin3, IP-10, MCP-1, MCP-4, MDC, MIP-1 α , MIP-1 β , and TARC. Briefly, after blocking the plate well, the serum is added and incubated for 2 hours and chemiluminescence determined using the Sector Imager Reader (Meso Scale Discovery).

Statistical analysis

Prior to analysis, RFU values from SOMAscan™ assays were normalized against hybridization control sequences to correct for any systematic effects introduced during hybridization. Median normalization was performed across samples within an array. Calibration was performed using

samples in each array to allow comparison across runs.³⁵ To reduce heteroscedasticity, values were log2 transformed. Protein expression was modeled using a linear model with age as numerical covariate and disease as factor covariate; parameters were estimated using the empirical Bayes method (Smyth, 2004) available in the *Limma* package in R.³⁶ This approach allows us to obtain estimates of disease effect, adjusting for the potential impact of age. The age-coefficients can be used to adjust the protein expression accordingly. Default parameters were used, except the “proportion” parameter was set to 0.05. Between groups comparisons were assessed using contrast and the p-values of the moderated t-test were adjusted by the Benjamini–Hochberg procedure which controls the false discovery rate (FDR). Differentially expressed proteins were defined as those with fold change (FCH) ≥ 1.5 and FDR ≤ 0.05 . Correlation analysis was performed with Pearson correlation test or Spearman correlation test (when normal distribution of the data cannot be guaranteed). Results were summarized using Venn diagrams and heatmaps using R capabilities. Scatter plots and correlation plots were generated with GraphPad Prism6. Functional annotation of protein targets are performed using DAVID (<https://david.ncifcrf.gov/tools.jsp>) and Ingenuity Pathway Analysis (<http://www.ingenuity.com/products/ipa>).

RESULTS

We evaluated the reproducibility of the SOMAscan™ platform by comparing the expression of eight proteins with established ECL Plex fluorescent western blotting assays in sera taken from 20 subjects with AD.³⁴ IgE, thymus- and activation-regulated chemokine (TARC), macrophage-derived chemokine (MDC), and interferon- γ -inducible protein 10 (IP-10) showed a Spearman correlation coefficient >0.9 between ECL Plex fluorescent western blotting assays and SOMAscan™ assays ($P < 0.05$). Eotaxin, monocyte chemoattractant protein 1 (MCP-1), MCP-4 and IL-22 showed a Spearman correlation coefficient >0.5 ($P < 0.05$). Macrophage Inflammatory Protein 1a (MIP-1a) was below the limit of detection in 12 out of 20 subjects in ECL Plex fluorescent western blotting assays, and therefore did not show significant correlation between the two technologies (Table E2).

Inflammatory Skin Diseases Show Common and Distinct Proteomic Profiles

Serum proteins in AD, CD, and PS were compared with HC. Overall, 66 proteins showed significant upregulation (FCH ≥ 1.5 and FDR ≤ 0.05) in at least one of the diseases (Figure 1A, 1C, Table E3) and 64 proteins showed significant downregulation (FCH ≥ 1.5 and FDR ≤ 0.05) [Figure 1B, 1D, Table E3]. Complement C5A anaphylatoxin (C5A), Lipopolysaccharide binding protein (LBP), C-reactive protein (CRP), Leukocyte immunoglobulin-like receptor subfamily B member 2 (ILT-4) and Sialic acid-binding Ig-like lectin 14 (SIG14) were found to be upregulated in all three diseases (Fig 2A) suggesting a common inflammatory signature in circulation as evidence of significant skin barrier disruption. No protein was detected to be commonly down-regulated.

Proteomic Signature Identification in Atopic Dermatitis

SOMAscan™ detected 14 proteins with significant upregulation and 14 proteins with significant downregulation in AD ($FCH \geq 1.5$, $FDR \leq 0.05$), compared with HC (Figure 1A, 1B, Table E3). The proteomic signature for AD was refined by comparing proteomic modulation with CD and PS. IgE, TARC, and MDC showed significant upregulation in AD alone compared with CD and PS (Fig 3A, Table E4), suggesting these three inflammatory mediators as a signature of AD. No unique proteins were found to be significantly down-regulated in AD alone.

As mentioned above, the detection of TARC, MDC and IgE by SOMAscan platform was confirmed by ECL Plex fluorescent Western blotting from serum samples of the same AD subjects. For all three proteins, the Spearman correlation coefficient between the two platforms was approximately 0.97 ($P < 0.05$) (Fig 3B and Table E2).

We further explored the relationship between expression of these three proteins and SCORAD disease severity. TARC and MDC both showed significant correlation with SCORAD (TARC: Spearman correlation $r = 0.57$, $P < 0.05$; MDC: $r = 0.52$, $P < 0.05$), while IgE showed a slightly weaker correlation ($r = 0.49$, $P < 0.05$) (Fig 3C). Approximately 70–80% of subjects with AD have elevated serum IgE concentrations (≥ 200 kU/L; extrinsic AD) and 20–30% have normal concentrations of serum IgE (< 200 kU/L; intrinsic AD). Previously it was shown that patients with extrinsic AD have a stronger correlation between serum IgE and disease severity.³⁷ Our cohort of subjects with AD was comprised of 14 extrinsic and 6 intrinsic AD. Consistent with previous observations,³⁷ the association between IgE and SCORAD was stronger in extrinsic ($r = 0.75$, $P < 0.01$) than in intrinsic subjects ($r = 0.49$, $P = 0.03$) (Fig 3C). Furthermore, TARC and MDC, but not IgE, significantly correlated with epidermal thickness, an indication of AD disease severity ($r = 0.71$ for TARC, $r = 0.74$ for MDC, $P < 0.01$) (Fig E1).

Proteomic Signature Identification in Contact Dermatitis

Proteomic analysis detected 24 proteins with significant upregulation and 40 proteins with significant downregulation in CD compared with HC (Figure 1A, 1B, Table E5). We further refined the protein signature associated with CD by comparing with AD and PS to detect significant upregulation of Bone morphogenetic protein 14 (BMP-14), BMP-7, Lactadherin (MFGM), Polymeric immunoglobulin receptor (PIGR), Albumin, $\alpha 2$ -Macroglobulin and Growth/differentiation factor 2 (GDF2) (Fig 4A and Table E5) and down-regulation of 14 proteins outlined in Figure E2 and Table E5.

KYNU Modulation is Unique to Psoriasis

Kynureninase (KYNU), lectin galactoside-binding soluble 3-binding protein (LG3BP), and Tryptase beta-2 (TPSB2) were significantly increased, and Carbonic anhydrase 6 (CA6) was significantly decreased in PS compared with HC (Table E6). Among these proteins, KYNU also showed significant upregulation when comparing PS with AD and CD (Fig 4B and Table E6) suggesting its utility as a biomarker for PS. KYNU expression has been reported to be upregulated in psoriasis in multiple

transcriptomic studies and was reported as one of the 20 signature genes that discriminate between lesion and non-lesion skin.³⁸⁻⁴³ Our study demonstrates that increased KYNU in circulation can also discriminate individuals with active PS. Interestingly, no significant correlation was observed between concentrations of KYNU and disease PASI score ($P=0.67$, $r=0.14$).

Novel proteins are associated with subjects with both AD and contact dermatitis

In our study, 10 subjects had clinical manifestations of CD and a history of AD (AD+CD). Given the heterogeneity of disease, we compared protein concentrations of the subjects with both AD+CD with HC (Figure 5 A–C). The proteomic profile in AD+CD subjects was similar to subjects with CD, while a greater level of protein dysregulation was observed (Table E7 and Fig 5C). A total of 68 proteins showed significant upregulation in subjects with AD+CD and 86 proteins showed significant downregulation in subjects with AD+CD (Figure 5B, 5C). Twenty-one proteins that were upregulated in AD+CD did not show significant upregulation in either AD or CD (Fig 5A, 5C). Several of these proteins are involved in immune cell activation, signaling, and trafficking, such as bactericidal permeability-increasing protein (BPI), complement component 1r subcomponent (C1r), granulocyte colony-stimulating factor (G-CSF), drebrin-like protein (DBNL), fatty acid binding protein 3 (FABP3), heterogeneous nuclear ribonucleoproteins A2/B1 (HnRNP A2/B1), soluble intercellular adhesion molecule 5 (sICAM5), IL13 receptor A (IL13 Ra1), macrophage migration inhibitory factor (MIF), myeloperoxidase, moesin, proteinase-3, and tumor necrosis factor receptor superfamily member 12A (TWEAKR), indicating an exacerbated immune response. Thirty-one proteins that were downregulated in AD+CD did not show significant downregulation in either AD or CD.

DISCUSSION

AD, PS, and CD are skin diseases characterized by significant barrier disruption, epidermal inflammation, abnormal immune activation, and cellular activation. Current diagnosis primarily involves the historical features of patients and appearance of skin lesions. In some instances, similarities make it challenging to distinguish between the diseases. Previous approaches to characterize and differentiate these diseases focused on transcriptomic profiling of skin biopsies.^{18, 44, 45} While this increased our mechanistic understanding of each disease, the invasive nature of skin biopsies prevents it from being incorporated into routine clinical practice. Additionally, upregulation of gene transcript does not always result into translation of protein. Recent studies demonstrate that these diseases are characterized by systemic inflammation which may provide the opportunity to non-invasively distinguish diseases through evaluation of circulating proteomic signatures.⁴⁴⁻⁴⁶

Using broad proteomic analysis of sera from well-characterized subject cohorts, we identified unique proteomic signatures for AD, CD, and PS. Interestingly, several proteins were elevated in all three skin diseases suggesting that local barrier disruption may trigger similar inflammatory activation in circulation. Specifically, a circulating signature of PARC (CCL18), C5A, LBP, CRP, ILT-4, and SIG14 was identified in all three diseases. PARC is a dendritic cell-derived chemokine upregulated in AD that mediates skin homing of human memory T cells.⁴⁷⁻⁴⁹ Furthermore, significant decrease of PARC was observed in AD after immunotherapy.^{50, 51} Increased gene expression of PARC

was reported in PS, although to a lesser extent than AD.⁵² This is consistent with our observations; we also showed upregulation of PARC in CD. CRP is produced in response to inflammation. Increased plasma concentrations of CRP have been reported in patients with PS, and were significantly reduced by etanercept treatment.⁵³⁻⁵⁵ In addition to PS, we observed increased levels of CRP in AD and CD in the current study. C5A, LBP, SIG14, and ILT-4 have not been previously associated with AD, PS, or CD. Complement proteins play important roles in inflammatory and cell killing processes by bridging the innate and adaptive immune responses. Interestingly, the TRAF1-C5 locus has been identified in multiple studies as a genetic risk factor for autoimmune diseases such as rheumatoid arthritis, type I diabetes, and systemic lupus erythematosus.⁵⁶⁻⁶⁰ LBP is involved in the acute-phase immunologic response to gram-negative bacterial infections and shows increased concentrations in multiple autoimmune diseases, including arthritis and chronic obstructive pulmonary disease.^{61, 62} SIG14 is a paired receptor with SIG5 and is believed to mediate signaling in neutrophils and macrophages in innate immune responses.⁶³ Interestingly, ILT-4 is generally considered an immune inhibitory factor that mediates the function of regulatory and suppressive T cells;⁶⁴⁻⁶⁸ the mechanism of its upregulation and function in these diseases remains to be explored. Overall, upregulation of these proteins in all three diseases supports the critical role of the innate immune system in the pathogenesis of these diseases.

Although the pathogenic mechanisms are not fully understood, these skin diseases are traditionally characterized by differential inflammatory pathways. Th2 and Th22 cells play a critical role in AD development, supported by increased blood eosinophilia and enhanced serum IgE concentrations. Interestingly, IL-4, IL-5, and IL-13 were not significantly elevated in AD alone. Previous publications have shown significant elevations of these Th2 cytokine genes in lesional AD skin;^{21,37} however, there is conflicting information on the levels of these cytokines in circulation of adult AD patients as many platforms lack sensitivity necessary for accurate measurements. Recently, periostin was identified as a potential surrogate for IL-4 and IL-13 signaling and activation.⁹² In our study, we observed an increase in periostin between AD and HC with a trend towards significance ($p=0.057$) confirming previous observations of the Th2 axis.

TARC (CCL17) and MDC (CCL22) are two major pro-Th2 mediators. Both TARC and MDC are specific ligands for CCR4, which is preferentially expressed on T cells, especially the Th2 subset.⁶⁹⁻⁷¹ CCR4+ TH2/TC2 cells have been shown to be expanded in severe AD, and CCR4 plays a critical role to mediate the homing of Th2 cells to the lesion skin.^{22,72} TARC and MDC have been reported to be upregulated in patients with AD.⁷³⁻⁷⁶ Our study confirmed the upregulation of IgE, TARC, and MDC protein concentrations in the serum of subjects with AD, and suggest these proteins as a signature to distinguish AD from other inflammatory skin diseases. While the concentrations of these proteins correlate with disease severity (SCORAD), their gene expression levels did not correlate, highlighting that serum proteins may better reflect disease progression and more accurately identify those with AD.

Seven proteins were detected to be upregulated in CD compared to HC, AD and PS. Among these, α 2-Macroglobulin and Albumin are both abundant plasma proteins. Leakage of plasma proteins is a hallmark of allergy and inflammation due to increased permeability of the vasculature.

In asthmatic patients, significant increase of α 2-Macroglobulin and Albumin in BAL fluid and serum were observed after segmental allergen challenge.⁷⁷⁻⁸¹ α 2-Macroglobulin increase was also reported in persistent allergic rhinitis.⁸² Although originally characterized as a proteinase inhibitor, α 2-Macroglobulin also functions as an antigen-delivery vehicle. It binds to bioactive peptides and is then internalized by macrophage and other antigen presentation cells, therefore enhances the cytotoxic T lymphocyte response.⁸³ Increase of these 2 proteins supports the allergic nature of CD. Interestingly, BMP-14, BMP-7 and GDF2 are all TGF-beta super family members. Although TGF β is generally considered anti-inflammatory⁸⁴, a recent study showed that^{85, 86} TGF β 1 can promote induction of Th17 cells in the presence of IL-6, as well as alter homeostasis of multiple cutaneous dendritic cell subsets, which may enhance skin inflammation.

KYNU is upregulated in PS compared to HC, as well as AD and CD. KYNU is a key enzyme in the kynurenine pathway, which catalyzes the hydrolysis of 3-hydroxy-l-kynurenine to produce 3-hydroxyanthranilic acid and l-alanine. Although the mechanisms are not fully understood, the kynurenine pathway has been shown to play an important role in inflammation. Metabolites downstream of KYNU have been shown to induce pro-inflammatory cytokines and chemokines, such as IL20, IL8, CCL2 and E-selectin in keratinocytes and endothelial cells. The gene expression of KYNU was reported to be upregulated in PS and regulated by the Th17 cytokine IL17 and the Th1 cytokine TNF α synergistically.⁸⁷ Furthermore, immunohistochemistry assays has revealed that lesional psoriatic skin is associated with increased number of dermal cells⁸⁸ expressing KYNU. Our discovery supports that kynurenine metabolism may play an important role in PS pathogenesis and suggests KYNU levels in circulation can distinguish PS from other inflammatory skin diseases. Previous reports have described a greater prevalence of obesity in patients with PS compared with AD.⁸⁹ In this study, we observed increased body mass index (BMI) scores in the PS patients compared with AD. Therefore, the observed elevation may be due to an increase in BMI rather than disease pathogenesis, although no significant correlation was observed between KYNU and BMI in PS patients (data not shown).

AD and CD are both characterized by significant epidermal barrier disruption and epithelial inflammation. Recently, several studies have demonstrated overlapping transcriptomic signatures in the lesional skin of AD and CD subjects.^{30,44} Additional characterization by the investigators identified unique transcriptomic profiles in the skin that corresponded with different sensitization profiles. In our cohort, 10 subjects displayed symptoms of both AD and CD, which accounts for 33% of all patients with AD and 50% of all patients with CD. Given the potential differences in their sensitization profiles, we hypothesized that subjects with both AD and CD would have a distinct proteomic signature independent from both AD and CD. Importantly, the subjects with AD and CD display novel dysregulated proteins,^{90, 91} especially upregulation of immune-related proteins.

In conclusion, we present the first high-throughput proteomic profiling across AD, PS, and CD using SOMAscan™ proteomic assays. While these diseases share common protein dysregulation, analysis revealed distinct serum protein expression patterns. Using broad proteomic approaches, we

identified proteins associated with each disease. Unique proteomic signatures may be valuable for correct disease diagnosis and personalized treatment. SOMAscan provides the opportunity to evaluate >1000 proteins from limited amount of serum samples. However, the specificity and sensitivity of the platform remains to be fully evaluated and caution needs to be taken when interpreting the result. Similar to microarrays, the quality of the platform relies heavily on design of the probes. While we observed increased levels of TARC, MDC and IgE, which are hallmarks of Th2 response, we did not observe significant increases in IL-4, IL-5, or IL-13 in AD compared with the other cohorts. Other pro-inflammatory molecules, such as IFN- γ , IL-17A and TNF- α , did not show significant difference between disease subjects and healthy controls. As more proteins are added to this and other assays, it will be important to confirm the sensitivity of both the assay and targets using independent approaches. This will further validate the identification and use of proteomic signatures with patient populations. It will also be important to extend the analysis to larger patient populations and perform mechanistic studies to understand the function of these proteins in disease pathogenesis.

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FIGURE LEGENDS

FIG 1. Protein targets with significant expression change in atopic dermatitis (AD), contact dermatitis (CD), and psoriasis (PS), compared with healthy controls (HC).

(A-B) Venn diagram showing overlap of proteins with significant upregulation (A) and downregulation (B) ($FCH \geq 1.5$ and $FDR \leq 0.05$) in AD, CD, and PS compared with HC. (C–D). Heatmap showing expression of proteins with significant upregulation (C) and downregulation (D) in at least one of the diseases compared with HC ($FCH \geq 1.5$ and $FDR \leq 0.05$). Red indicates high expression and blue indicates low expression. Number of subjects: N=20 for AD, N=10 for CD, N=12 for PS, N=10 for HC.

FIG 2. Atopic dermatitis (AD), contact dermatitis (CD), and psoriasis (PS) share common dysregulated proteins compared with healthy controls (HC). (A) Scatter dot plot showing expression of proteins with significant upregulation in all three diseases compared with HC. (B) Scatter dot plot showing expression of proteins with significant downregulation in all three diseases compared with HC. Y axis indicates $\log_2$ transformed signal intensity from SOMAscan™ assays (relative fluorescence units [RFU]). Lines indicate group mean with standard deviation. *False discovery rate (FDR) ≤ 0.05 , **FDR ≤ 0.01 , ***FDR ≤ 0.001 . Number of subjects: N=20 for AD, N=10 for CD, N=12 for PS, N=10 for HC.

FIG 3. IgE, TARC, and MDC showed significant upregulation in atopic dermatitis (AD) compared with contact dermatitis (CD), psoriasis (PS), and healthy controls (HC), and significant correlation to SCORAD. (A) Scatter dot plot showing expression of IgE, TARC, and MDC in each group. Y axis indicates $\log_2$ transformed signal intensity from SOMAscan™ (relative fluorescence units [RFU]). Lines indicate group mean with standard deviation. *False discovery rate [FDR] ≤ 0.05 , **FDR ≤ 0.01 , ***FDR ≤ 0.001 . Number of subjects: N=20 for AD, N=10 for CD, N=12 for PS, N=10 for HC. (B) Spearman correlation between $\log_2$ transformed SOMAscan™ signal intensity and serum protein levels (kU/L) for IgE measured by ELISA, $\log_2$ transformed values in units of pg/mL for TARC and MDC measured by ECL Multiplex. r: Spearman correlation coefficient. p: P value for Spearman correlation test. Number of subjects: N=16. (C) Spearman correlation between $\log_2$ transformed signal intensity of IgE, TARC, and MDC and SCORAD of AD patients. For IgE, dashed line shows correlation with all values from all patients with AD; solid line shows correlation with values from patients with extrinsic AD. r: Spearman correlation coefficient. p: P value for Spearman correlation test. Number of subjects: N=20.

FIG 4. Protein targets showed significant dysregulation specifically in contact dermatitis (CD) and psoriasis (PS). (A) Scatter dot plot showing expression the 10 proteins with significant upregulation in CD compared with AD, PS, and HC. (B) Scatter dot plot showing expression the kynureninase (KYNU) protein with significant upregulation in PS compared with AD, CD, and HC. Y axis indicates $\log_2$ transformed signal intensity from SOMAscan™. Lines indicate group mean with standard deviation. *False discovery rate (FDR) ≤ 0.05 , **FDR ≤ 0.01 , ***FDR ≤ 0.001 . Number of subjects: N=20 for AD,

N=10 for CD, N=12 for PS, N=10 for HC.

FIG 5. Protein targets showed significant expression change in patients with both atopic dermatitis and contact dermatitis (CD) compared with healthy controls (HC). (A-B) Venn diagram showing overlap of proteins with significant upregulation (A) and downregulation (B) in AD+CD, AD, and CD compared with HC. (C) Heatmap showing expression of proteins with significant upregulation or downregulation in AD+CD, but not in AD or CD, compared with HC. Red indicates high expression and blue indicates low expression. Number of subjects: N=10 for AD+CD, N=20 for AD, N=10 for CD, N=10 for HC.

Figure 1

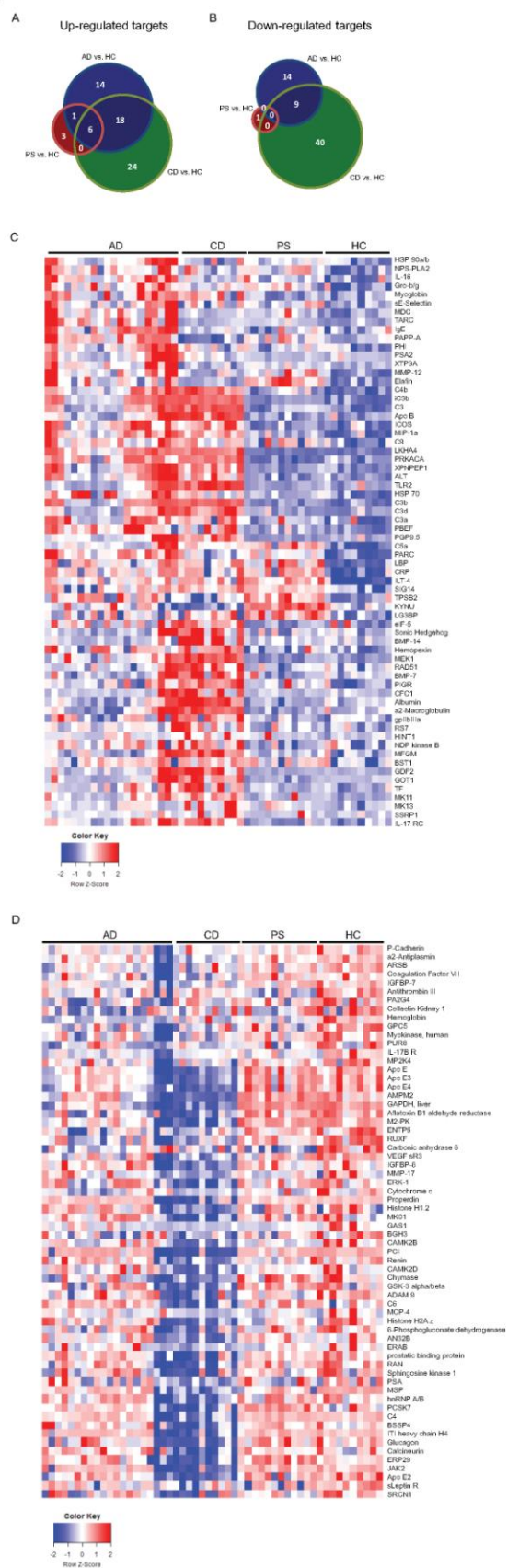


Figure 2

A

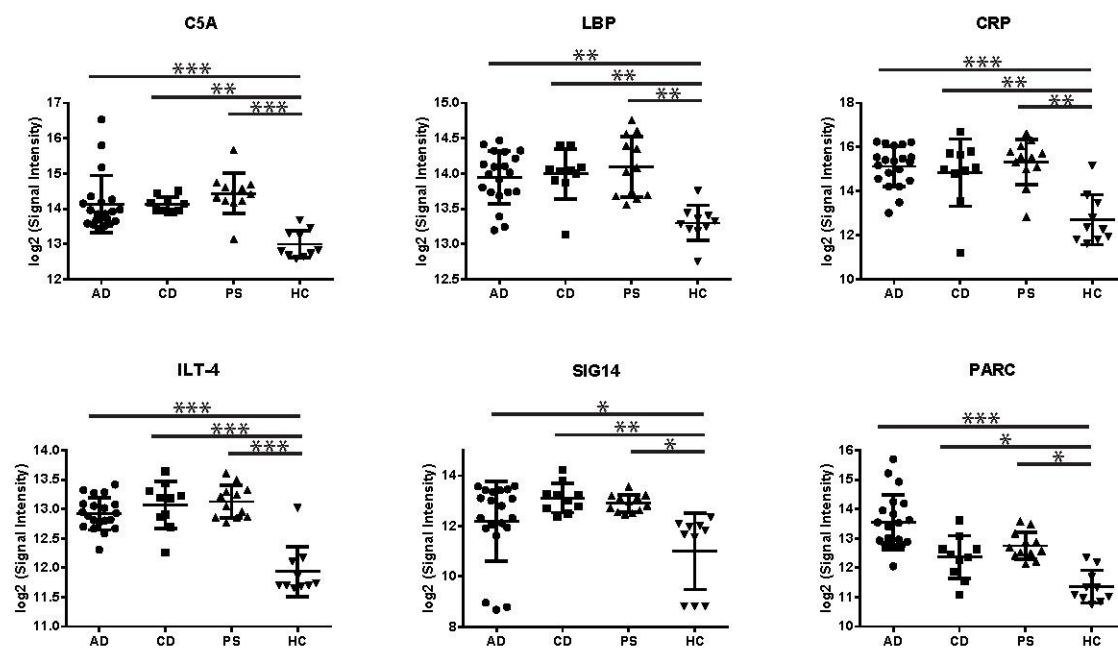
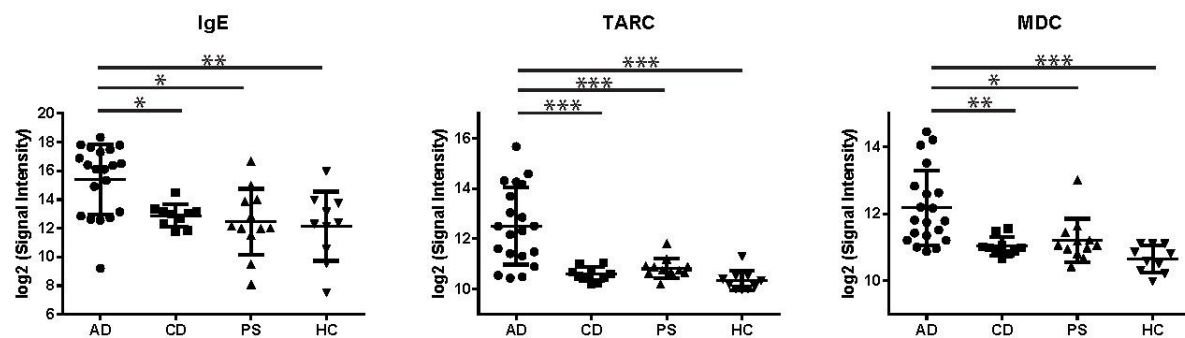
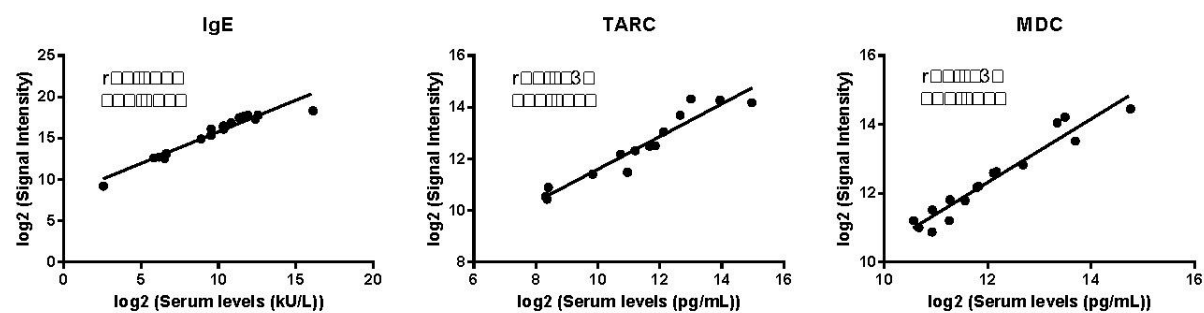


Figure 3

A



B



C

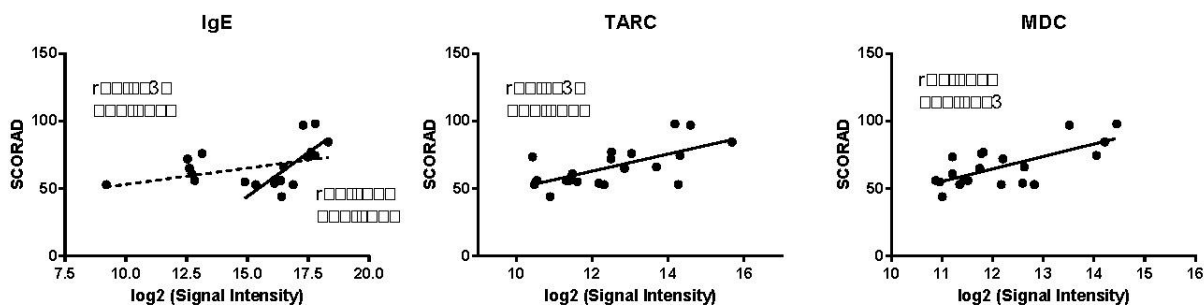
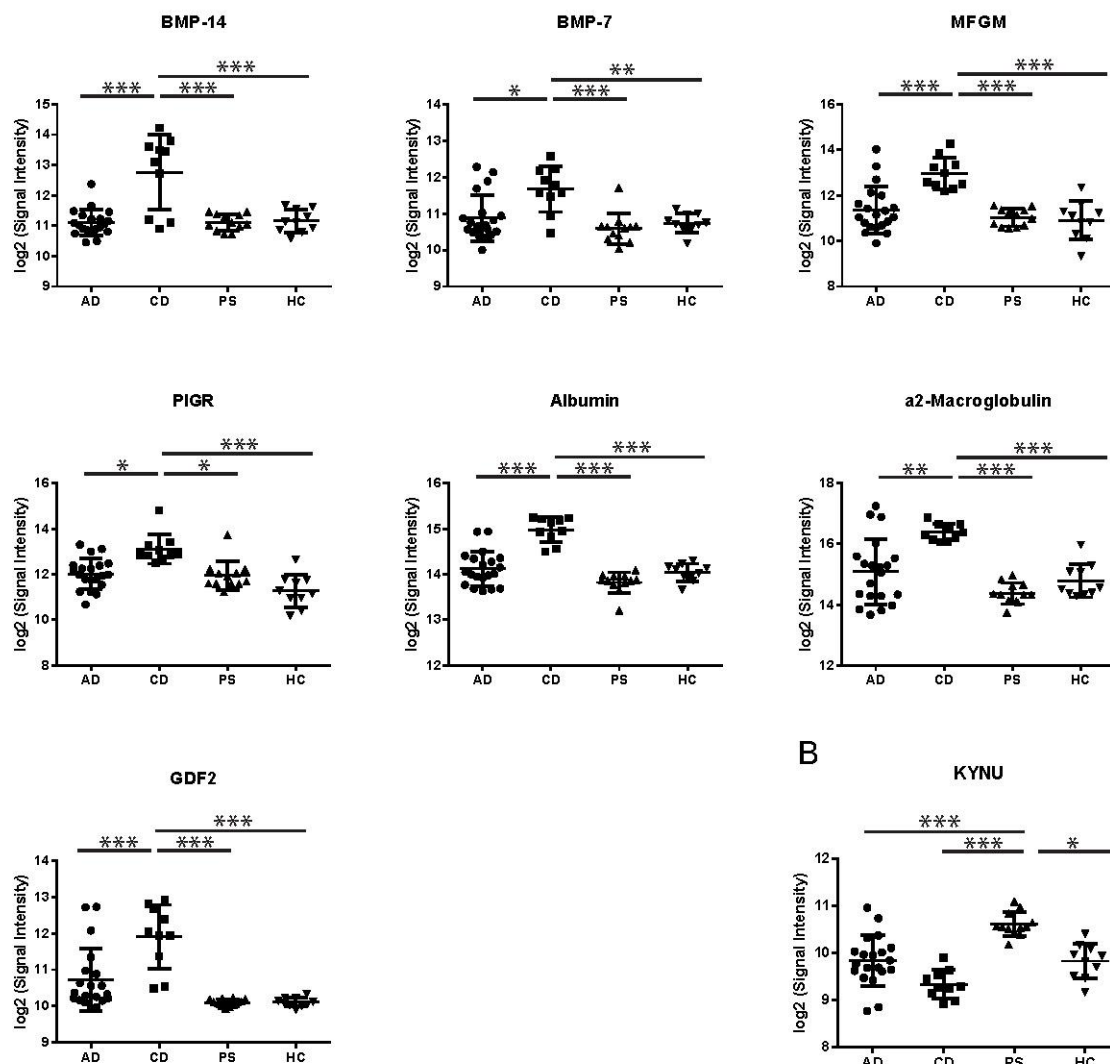


Figure 4

A



B

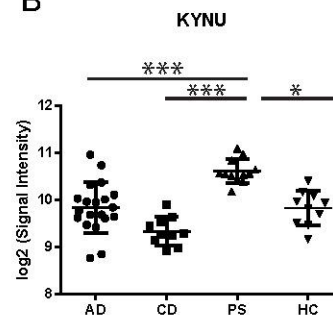


Figure 5

